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*J Immunol* 2009; 183:7307-7313; Prepublished online 16 November 2009; doi: 10.4049/jimmunol.0902736

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TGFβ Neutralization within Cardiac Allografts by Decorin Gene Transfer Attenuates Chronic Rejection

Susan M. Faust, Guanyi Lu, Sherri C. Wood, and D. Keith Bishop

Chronic allograft rejection (CR) is the leading cause of late graft failure following organ transplantation. CR is a progressive disease, characterized by deteriorating graft function, interstitial fibrosis, cardiac hypertrophy, and occlusive neointima development. TGFβ, known for its immunosuppressive qualities, plays a beneficial role in the transplant setting by maintaining alloreactive T cells in a hyporesponsive state, but has also been implicated in promoting graft fibrosis and CR. In the mouse vascularized cardiac allograft model, transient depletion of CD4+ cells promotes graft survival but leads to CR, which is associated with intra-graft TGFβ expression. Decorin, an extracellular matrix protein, inhibits both TGFβ bioactivity and gene expression. In this study, gene transfer of decorin into cardiac allografts was used to assess the impact of intra-graft TGFβ neutralization on CR, systemic donor-reactive T cell responses, and allograft acceptance. Decorin gene transfer and neutralization of TGFβ in cardiac allografts significantly attenuated interstitial fibrosis, cardiac hypertrophy, and improved graft function, but did not result in systemic donor-reactive T cell responses. Thus, donor-reactive T and B cells remained in a hyporesponsive state. These findings indicate that neutralizing intra-graft TGFβ inhibits the cytokine’s fibrotic activities, but does not reverse its beneficial systemic immunosuppressive qualities. The Journal of Immunology, 2009, 183: 7307–7313.

The Journal of Immunology

Received for publication August 19, 2009. Accepted for publication October 5, 2009.

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1 This work was supported by R01 HL070613 (to D.K.B.) and by R01 AI061469 (to D.K.B.) and by T90 DK070071 (to S.M.F.).
2 Address correspondence and reprint requests to Dr. D. Keith Bishop, Transplant Immunology Research Laboratory, Section of General Surgery, A560 MSRB II, Box 0654, University of Michigan Medical Center, Ann Arbor, MI 48109. E-mail address: kbishop@umich.edu
3 Abbreviations used in this paper: CR, chronic rejection; Treg, T regulatory cell; ECM, extracellular matrix; WT, wild type; AdDec, adenovirus that encodes decorin; AdΔgal, adenovirus that encodes β-galactosidase; CD4-DNR, transgenic mice with a dominant negative TGFβ receptor.
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5 www.jimmunol.org/cgi/doi/10.4049/jimmunol.0902736
distinct from naive cells in that repopulating CD4+ are hyporesponsive toward the graft but mount Th2 recall responses (34). The importance of TGFβ in allograft acceptance and suppression of graft-reactive T and B cells was revealed in transplant experiments using mice with a dominant negative TGFβRII transgene (CD4-DNR) (73), which render the animals unresponsive to T cell TGFβ signaling (35). Transient CD4+ T cell depletion of CD4-DNR recipients resulted in both alloreactive cellular and humoral responses, which remained hyporesponsive in wild-type (WT) recipients indicating that TGFβ is critical to suppression of T and B cell responses in this system. Graft rejection in these recipients correlated with CD4+ T cell repopulation of the periphery (73). These studies revealed that inductive anti-CD4 mAb treatment is a TGFβ-dependent model of allograft acceptance and that IL-17 is a critical element in TGFβ-driven fibrosis (73).

In this study, we used decorin gene transfer into cardiac allografts to assess the impact of intragraft TGFβ neutralization on CR, graft function, donor-reactive T and B cell responses, and allograft acceptance. We demonstrate that neutralizing intragraft TGFβ inhibits the cytokine’s fibrotic activities, but does not reverse its beneficial systemic immunosuppressive qualities.

Materials and Methods

Mice

C57BL/6 WT (H-2b) and BALB/c (H-2d) mice were purchased from The Jackson Laboratory. All mice were housed under specific pathogen-free conditions in the Unit for Laboratory Animal Medicine at the University of Michigan. These experiments were approved by the University Committee on Use and Care of Animals at the University of Michigan.

Culture medium

Culture medium consisted of the following: RPMI 1640 supplemented with 2% FCS, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 1.6 mM L-glutamine, 10 mM HEPEs buffer (all from Invitrogen), 0.27 mM t-aspartagine, 1.4 mM t-arginine HCl, 14 μM folic acid, and 50 μM 2-ME (all from Sigma-Aldrich).

Vascularized cardiac transplantation

C57BL/6 mice were transplanted with intact BALB/c cardiac allografts, as described (36). In brief, the aorta and pulmonary artery of the donor heart were anastomosed end-to-side to the recipient’s abdominal aorta and inferior vena cava, respectively. Upon perfusion with the recipient’s blood, the transplanted heart resumes contraction. Graft function is monitored by abdominal palpation.

Adenoviral-mediated transduction of cardiac allografts

As described (37, 38), cardiac allografts were transduced by perfusion via the aorta with E1-E3 deleted adenoviral vectors (5 × 10^10 pfu) encoding rat decorin (AdDec) or β-galactosidase (Adβgal). AdDec was constructed in Dr. Elizabeth Nabel’s laboratory using a cDNA provided by Dr. Wayne Border (University of Utah, Salt Lake City, UT) (26). Following perfusion, grafts were recovered and placed in iced ringers for 1 h before transplantation. Reporter gene studies with Adβgal have revealed that the distribution of transgene expression within the cardiac allograft is patchy, and that both cardiac myocytes and cells of the vasculature express the transgene product (37).

In vivo mAb treatment

The hybridoma secreting anti-CD4 mAb (clone GK1.5) was obtained from American Type Culture Collection. Anti-CD4 was purified and resuspended in PBS by Bio X Cell. Mice received 1 mg i.p. of anti-CD4 mAb on days −1, 0, and 7 (21, 31, 32). All doses are relative to day of transplant.

Histology

Allografts were recovered at the times indicated posttransplantation, fixed in formalin, and embedded in paraffin. Sections were stained with H&E to assess myocyte viability (presence of cross striation and myocyte nuclei), and the nature and intensity of graft infiltrating cells.

Morphometric analysis of cardiac allograft fibrosis and hypertrophy

Graft fibrosis was quantified by morphometric analysis of Masson’s trichrome stained tissues using iPLab software (Scalinitics) (3). Masson’s trichrome stains fibrotic tissue blue. Mean fibrotic areas were calculated from 10 to 12 areas per heart section analyzed at ×200 magnification. Nine individual hearts were analyzed per group. To quantify cardiomyocyte area as a measure of hypertrophy, digital outlines were drawn around 100 cardiomyocytes from views of H&E stained grafts at ×200 magnification. Areas within outlines were quantified using SCION IMAGE β 4.0.2 software (Scion Corporation) to measure cardiomyocyte cell size (3). Five individual hearts were analyzed per group.

ELISPOT assays for cytokine-producing cells

ELISPOT assays were performed as previously described (39). Capture and detection mAbs specific for IFN-γ (R-6–62A, XMG1.2), IL-4 (11B11, BV6–24G2), and IL-17 (TC11-18H10.1, TC11-8H4.1) were purchased from BD Pharmingen. Polyvinylidene difluoride-backed microtiter plates (Millipore) were coated with unlabelled mAb and blocked with 1% BSA in PBS. Irradiated (1000 rad) donor splenocytes (4 × 10^6) and 1 × 10^6 recipient splenocytes were added to the plates. After washing, a 1/1000 dilution of anti-biotin alkaline phosphatase conjugate (Vector Laboratories) was added to IFN-γ and IL-17 plates, and a 1/2000 dilution of HRP-conjugated streptavidin (SA-HRP; DakoCytomation) was added to IL-4 plates. Plates were washed and spots visualized by addition of NBT (Bio-Rad)/3-bromo-4-chloro-inoyl-phosphate (Sigma-Aldrich) to IFN-γ and IL-17 plates, or 3-aminoo-9-ethylcarbazole (Pierce) to IL-4 plates. Color development continued until spots were visible and stopped by adding H2O. Plates were dried and spots quantified with an Immunoscan Series 1 ELISPOT analyzer (Cellular Technology).

RNA isolation and quantitative RT-PCR

Cardiac allografts were homogenized in 1 ml TRIzol (Invitrogen Life Technologies) and RNA was isolated as per manufacturers protocol. 5 μg of total RNA were reverse transcribed using ×10 PCR buffer (Roche), 10 mM dNTPs, Oligo (dt), M-MLV-RT (all from Invitrogen), and RNasin (Promega). Products were then cleaned with 1:1 phenol/chloroform isoamyl (25:24:1) and re-precipitated with 7.5 M NH4OAC in pure EtOH overnight at −80°C.

Quantitative RT-PCR was performed on cDNA using a Rotor-Gene 3000 TM (Corbett Life Science, CA). Primer binding to DNA was detected by SYBR Green I™ dye (Roche, Indianapolis, IN). Relative expression of the gene of interest was calculated by accompanying Rotor-Gene software and the concentration of the gene product compared with GAPDH. Significance was determined with an unpaired Student t test.

Primer sequences: Rat Decorin sense: 5’-AGCATAAATATGTGCCG TGTTCG; Rat Decorin anti-sense: 5’-GGAATTCTTCTGATGTTG CAATGA; TGFβ sense: 5’-CTCTGTTGACCGTCTAGTA TGATAGG; TGFβ anti-sense: 5’-CTTGATTCCGTCCTTCTCTTGG T; GAPDH sense: 5’-CTTGATTCCGTCCTTCTCTTGG T; GAPDH anti-sense: 5’-GGAATTCTTCTGATGTTG CAATGA; IL-17 sense: 5’-GACCGAGATCTCTTGCCTGG A; IL-17 anti-sense: 5’-GACCGAGATCTCTTGCCTGG A; GAPDH sense: 5’-CTGTGTTGACTTGTATGTG CGT; GAPDH anti-sense: 5’-CGATTTCTGAGTGGCAATG CT.

Donor-reactive Ab determination

P815 cells (H-2b) were stained for flow cytometric analysis using diluted (1/50) sera obtained from mice as the primary Ab, followed by FITC-conjugated isotype specific anti-mouse IgG and IgM secondary Abs (The Binding Site) used at a 1/50 dilution (33). Data are reported as the mean channel fluorescence determined on a Becton Dickinson FACSCalibur.

Statistical analysis

Data were analyzed with GraphPad Prism 4.0c software using unpaired Student t tests. p values of ≤0.05 were considered statistically significant.

Results

Rationale

Prolonged allograft survival can be accomplished in the mouse cardiac allograft model by depleting CD4+ T cells transiently at the time of transplant. However, allografts in anti-CD4 mAb treated recipients develop interstitial fibrosis and CR, which is associated with intragraft TGFβ expression (21). TGFβ is beneficial...
In the transplant setting, and has been associated with the progression of donor-reactive T and B cells to a hyporesponsive state in recipients treated inductively with anti-CD4 mAb (34, 73). Conversely, T cell responsiveness to TGFβ can be deleterious for the graft by inducing fibrosis (73). Therefore, we explored the impact of local TGFβ neutralization using decorin gene transfer into the allografts. We assessed the impact of intragraft TGFβ neutralization on allograft acceptance, graft function, T and B cell hyporesponsiveness and CR.

Decorin gene transfer into cardiac allografts

Prior studies using adenoviral transduction of allografts revealed long-term transgene expression and negligible off-target tissue effects (21, 37). To evaluate efficacy of decorin gene transfer, allografts were transduced with AdDec or Adβgal and transplanted into recipients treated inductively with anti-CD4 mAb. Functioning allografts were harvested on day 7, 14, and 50 posttransplant (Fig. 1). Using rat decorin specific primers and quantitative RT-PCR, we verified that over-expression of rat decorin was detected predominantly within the cardiac allografts at day 7 and 14 and not within nontarget tissue, such as the spleen (Fig. 1A). In addition, long-term rat decorin gene expression was detected in the AdDec infected allografts and not in the βgal controls at day 50 posttransplant (Fig. 1B). These results demonstrate the efficacy and tissue localization of decorin gene transfer into allografts using adenoviral vectors, and the persistence of transgene expression.

Intragraft TGFβ neutralization by decorin gene transfer does not reverse T and B cell hyporesponsiveness in recipients treated with anti-CD4 mAb

Although TGFβ is a known profibrotic cytokine (40) it also has beneficial anti-inflammatory effects in the transplant setting and is frequently observed within accepted grafts (29, 30, 41, 42). Because TGFβ is critical in controlling donor-reactive responses following transient CD4+ T cell depletion (73), it was possible that intragraft neutralization of TGFβ might reverse immune hyporesponsiveness if decorin acted beyond the local confines of the allograft and inhibited TGFβ systemically (26). To determine whether localized TGFβ neutralization within the allografts affected systemic donor-reactive immune responses, ELISPOT was used to quantify the number of in vivo primed donor-reactive Th1 (IFN-γ), Th2 (IL-4), and Th17 (IL-17) responses (Fig. 2A). Gene transfer of βgal or decorin to allografts resulted in negligible T cell responses compared with untreated transplant recipients, indicating that TGFβ neutralization within cardiac allografts did not reverse graft-reactive T cell hyporesponsiveness in recipients depleted of CD4+ T cells (Fig. 2A). In addition, cardiac allograft contractions were noticeably stronger in AdDec transduced allografts when compared with βgal controls. These data demonstrate that intragraft inhibition of TGFβ had a beneficial effect on graft function and did not reverse systemic donor-reactive T cell hyporesponsiveness normally observed in recipients transiently depleted of CD4+ T cells.

TGFβ also inhibits B cell responses by affecting B cell proliferation, survival signals, activation, and IgG class switching (7). In CD4-DNR recipients treated with anti-CD4 mAb, T cells differentiate into effector cells and provide help to B cells, which produce donor-reactive IgG (73). To examine the effect of intragraft decorin over-expression on donor-reactive Ab, we quantified donor-reactive IgM and IgG production by flow cytometry (Fig. 2B). No difference in alloantibody production was observed in recipients whose allografts expressed decorin or βgal. This demonstrates that B cell hyporesponsiveness is also not reversed in recipients that overexpress decorin within their grafts. This further indicates that there are no systemic effects on the allogeneic immune responses by local TGFβ neutralization.

Effect of intragraft decorin gene transfer on TGFβ-induced gene expression

TGFβ is a pleiotropic cytokine that exerts a variety of effects on many different cell types. A reciprocal developmental pathway exists for the generation of pathogenic effector Th17 cells and Treg in response to TGFβ, with IL-6 being the cofactor required for Th17 induction (43–45). In addition, TGFβ induces cardiac fibroblasts to differentiate into myofibroblasts, which produce significant amounts of collagen and contribute to fibrosis (40). To assay for the effect of decorin on TGFβ-induced genes such as TGFβ, collagen A1, FoxP3 and IL-17, RNA was isolated from the allografts and quantitative RT-PCR was performed (Fig. 3). In recipients transduced with AdDec, intragraft TGFβ (p < 0.05), collagen A1 (p < 0.05), and IL-17 (p < 0.05) transcript levels were significantly reduced compared with control allografts (Fig. 3). In contrast, intragraft FoxP3 expression was comparable between AdDec and Adβgal transduced allografts. These observations indicate that localized TGFβ neutralization by decorin can significantly decrease gene expression associated with fibrosis, but does not.

FIGURE 1. Decorin gene transfer and gene expression. BALB/c allografts were transduced with AdDec or Adβgal and transplanted into C57BL/6 mice that were given inductive anti-CD4 therapy on days −1, 0, and 7 relative to transplant. Spleens and allografts were recovered on day 7 (light shaded bars) and day 14 (dark shaded bars) for AdDec transduced allografts (A). Functioning allografts were recovered on day 50 posttransplant for Adβgal (open bars) and AdDec (shaded bars) transduced allografts (B). Rat decorin expression relative to GAPDH was assessed by real-time RT-PCR. Bars represent the mean of RNA expression from a minimum of four Adβgal and four AdDec transduced allografts (±SEM).
affect FoxP3 expression. These data suggest Th17 polarization occurs within the site of inflammation, the graft, while Treg induction and maintenance occurs systemically in the secondary lymphoid tissues (46).

**Intragraft TGFβ neutralization significantly attenuates graft fibrosis and hypertrophy**

To investigate the effect of localized TGFβ neutralization on graft fibrosis, quantitative morphometric trichrome analysis was performed (Fig. 4). Assessment of allograft fibrosis revealed that intragraft decorin expression resulted in a significant reduction of collagen deposition compared with control grafts ($p < 0.01$) (Fig. 4, A and B). These data indicate that TGFβ promotes cardiac fibrosis and that localized neutralization of TGFβ can significantly attenuate CR.

**FIGURE 2.** Intragraft TGFβ neutralization does not reverse donor-reactive T and B cell hyporesponsiveness. A, On day 50 posttransplantation, splenocytes from Adβgal or AdDec transduced recipients were processed for ELISPOT assays to quantify primed, donor-reactive IFN-γ, IL-4, or IL-17-producing cells. C57BL/6 recipients of BALB/c allografts that received no treatment (striped bars) served as positive controls and splenocytes were harvested at the time of rejection. Bars represent the average number of cytokine producing cells (±SEM). Numbers in parentheses represent the number of recipients in each group. B, Fifty days posttransplant, sera were obtained from recipients transduced with Adβgal (open bars) or AdDec (shaded bars) and treated with inductive anti-CD4 mAb. P815 (H-2b) cells were incubated with of sera and bound donor-reactive Ab was detected by incubation with FITC-tagged anti-IgM or anti-IgG Abs. The mean channel fluorescence is indicative of the relative amount of donor-reactive Ab. Bars represent the average mean channel fluorescence of nine Adβgal or nine AdDec transduced recipient samples (±SEM).

**FIGURE 3.** Effects of TGFβ neutralization on intragraft gene expression. RNA samples from grafts of recipients transiently depleted of CD4+ T cells and transduced with Adβgal or AdDec were recovered 50 days posttransplant. Intragraft expression of TGFβ (A), collagen A1 (B), IL-17 (C), and FoxP3 (D) relative to GAPDH was assessed by real-time RT-PCR. Bars depict the means of RNA expression from nine Adβgal or eight AdDec transduced allografts (±SEM).

**FIGURE 4.** Decorin gene transfer and intragraft TGFβ neutralization attenuates fibrosis and hypertrophy. A, Sections of grafts from recipients treated with anti-CD4 and transduced with Adβgal or AdDec were stained with Masson’s trichrome stain on day 50 posttransplant. Fibrotic tissue appears blue. Magnification, ×200. B, Morphometric analysis of trichrome staining. Bars represent the average percentage (±SEM) of graft area positive for collagen in nine Adβgal (open bars) or nine AdDec (shaded bars) transduced allografts. C, Cardiomyocyte area quantification of groups described in A. Bars represent mean (±SEM) of area measurements from 100 cardiomyocytes per allograft at ×200 magnification. Five individual hearts were analyzed per group.
Cardiac hypertrophy is defined as an increase in the heart mass (47). An increase in the size of the cardiac myocytes, as opposed to the number, is the primary basis of cardiac hypertrophy (47). TGFβ is critical in driving this process (reviewed in Ref. 48). An up-regulation of TGFβ in cardiac tissue increases cardiomyocyte size and leads to cardiac dysfunction (48–50). To evaluate the effect of intragraft TGFβ neutralization on cardiac hypertrophy, cardiomyocyte cell size was measured using histologic analysis (3). Reduced cardiac hypertrophy was observed in AdDec transduced grafts (Fig. 4C). These findings indicate that intragraft TGFβ correlates with both fibrosis and hypertrophy in CR allografts and that decorin gene transfer can attenuate both pathologies.

**Discussion**

CR is an intractable disease characterized by interstitial fibrosis, occlusive neointima development, and graft dysfunction (4–6). The etiology of CR is poorly understood and no therapies exist to block its progression. TGFβ plays a beneficial role in the transplant setting because of its immunosuppressive qualities (29, 30), but has also been implicated in promoting graft fibrosis and CR (51, 52). In the mouse vascularized cardiac model, we have previously reported an association between TGFβ and CR (21). Intragraft TGFβ transcript levels were readily detected in the CR grafts from recipients transiently depleted of CD4+ T cells, but not in the grafts of anti-CD40L treated recipients, which remain free of CR (21), suggesting the importance of TGFβ in this pathology. In this study, we used decorin gene transfer and local neutralization of TGFβ in cardiac allografts to assess the impact of intragraft TGFβ neutralization on allograft acceptance, T and B cell hyporesponsiveness, and CR. We demonstrate that local neutralization of TGFβ in cardiac allografts significantly attenuated interstitial fibrosis and improved graft function, but did not reverse the hyporeactive state of donor-reactive T or B cells.

Intragraft transcript levels of TGFβ are frequently detected in accepted grafts, including the cardiac allografts from recipients transiently depleted of CD4+ T cells. TGFβ expression is believed to promote graft survival through the induction of Treg, which control graft-reactive Th1 and Th2 responses (29, 30, 41). Previous studies in recipients treated with inductive anti-CD4 mAb have revealed that repopulating CD4+ T cells are hyporesponsive toward donor Ag and mount Th2 responses upon rechallenge, while naïve T cells mount a dominant Th1 response (34). In this CR model, alloreactive T cells only progress to a hyporesponsive state in response to TGFβ (73). The critical role for TGFβ in this CR model was revealed when CD4-DNR mice were used as recipients and transiently depleted of CD4+ T cells. T cell TGFβ signaling was requisite for both long-term graft acceptance and suppression of graft-reactive T and B cell responses as well as graft fibrosis (73). Therefore, systemic strategies targeting TGFβ are not feasible because this could alter the hyporesponsiveness of graft-reactive T and B cells. In contrast, intragraft TGFβ inhibition would be beneficial in attenuating fibrosis. TGFβ neutralization within allografts, however, did not result in a reversal of T or B cell hyporesponsiveness (Fig. 2). These findings demonstrate that localized TGFβ inhibition does not alter the systemic regulation of graft-reactive cells or lead to graft loss but was effective at reducing fibrosis associated with CR.

In transplantation, FoxP3+ Treg have been shown to play a central role in suppression of alloreactive T cells and in long-term allograft acceptance (reviewed in Ref. 53). In both human and animal transplant, allograft acceptance strongly correlates with Treg infiltration into the graft as detected by enhanced intragraft FoxP3 transcript levels (53–56). Treg can be divided into two populations: natural Treg, which arise in the thymus and do not require TGFβ to develop, and induced peripheral Treg, which do require TGFβ to differentiate from naive CD4+ FoxP3+ T cells into Treg (reviewed in Ref. 57). In the current study, local neutralization of TGFβ did not affect intragraft FoxP3 expression, indicating that Treg migration and/or generation within the graft was unchanged between decorin and βgal transduced recipients (Fig. 3). However, these data argue against Treg induction within the grafts because intragraft TGFβ was neutralized by decorin gene transfer. Indeed, Treg have been reported to be generated in lymph nodes and subsequently migrate to the graft (46). Although thymic-derived Treg do not require TGFβ for their generation, they do depend on TGFβ for their persistence in the periphery (58). Given that decorin gene transfer spares the systemic effects of TGFβ, it is also possible that thymic-derived natural Tregs may contribute in regulating alloreactive responses within the graft (59).

Interstitial fibrosis represents a hallmark of CR and results in pathogenic cardiac remodeling and graft dysfunction (22). The effect of decorin gene transfer and local neutralization of TGFβ in cardiac fibroblasts, which respond to TGFβ by inducing the expression of profibrotic mediators that up-regulate extracellular matrix synthesis and down-regulate matrix degradation (22). During remodeling, cardiac fibroblasts located within the interstitium proliferate and produce proteins such as collagen (60), resulting in a significant increase in interstitial fibrosis (61). Cardiac fibrosis impairs contractility and reduces cardiac function.

Previous studies demonstrate that decorin gene transfer ameliorates TGFβ-induced fibrosis of multiple organs (26, 28). Decorin inhibits TGFβ bioactivity by sequestering TGFβ to the ECM (24, 25). In addition, decorin negatively impacts TGFβ gene expression by interrupting TGFβ/Smad-dependent transcriptional events (24, 25, 27). One mechanism by which decorin inhibits fibrosis is through the reduction of TGFβ-induced collagen transcript levels in cultured human cardiac fibroblasts (62). Gene transfer of decorin into allografts transplanted into recipients transiently depleted of CD4+ T cells significantly attenuated collagen deposition and fibrosis compared with control allografts (Fig. 4). Decorin overexpression also inhibited cardiac hypertrophy, demonstrating amelioration of an additional TGFβ-induced parameter correlated with CR (Fig. 4).

In addition to reduced TGFβ gene expression, decorin reduced intragraft transcript levels of IL-17 (Fig. 3). IL-17 amplifies inflammatory responses (reviewed in Refs. 63, 64) and has recently been identified as a cytokine with profibrotic activities (65–70). Prior studies in IL-17−/− recipients treated inductively with anti-CD4 mAb revealed that allografts from the deficient mice exhibited a significant reduction in fibrosis compared with WT (73). IL-17 may induce fibrosis though multiple mechanisms. Up-regulation of collagen gene expression in direct response to IL-17 has been observed in mouse cardiac fibroblasts (66). IL-17 may also induce endothelial cells and fibroblasts to secrete proinflammatory cytokines and chemokines (63, 64) that result in the recruitment of APC and alloreactive T cells into allograft. These inflammatory cells may secrete factors that lead to myocardial damage and tissue remodeling that favors fibrosis. The reduction of intragraft IL-17 expression in AdDec transduced grafts compared with WT counterparts further implicates this proinflammatory cytokine in CR.

Decorin has multiple molecular targets in cell growth in addition to its interaction with TGFβ (23). Decorin negatively impacts cell proliferation, an effect mediated through the induction of p21 (23). Decorin-induced cell cycle arrest might reduce fibrosis by suppressing cardiac fibroblasts from proliferating and differentiating into myofibroblasts. Decorin also interacts with complement C1q, inhibiting activation of the classical complement pathway (71).
Hence, under inflammatory tissue damage and ECM remodeling, decorin may suppress complement activation and prevent further cardiomyocyte injury. Decorin may further inhibit the production of inflammatory chemokines and cytokines, including MCP-1 and IL-8 by preventing C1q from binding graft endothelial cells (71, 72). Therefore, in addition to decorin’s inhibitory effects on TGFβ, suppression of complement activation may help to reduce fibrosis by limiting the damage inflicted on the allograft.

In summary, TGFβ is a critical cytokine in fibroproliferative disorders following inflammatory responses (reviewed in Ref. 16). TGFβ can have both exacerbating and ameliorating effects in immune-mediated fibrotic diseases, making global inhibition undesirable and local neutralization of TGFβ an attractive therapy. As evidenced in this model of CR, systemic TGFβ production is requisite for T cell hypersensitivity (73), while local TGFβ production at the site of inflammation induces graft fibrosis. We demonstrate that neutralizing intragraft TGFβ inhibits the cytokine’s fibrotic activities, but does not reverse its beneficial immunosuppressive qualities. These data provide insight into the underlying causes of CR, and identify intragraft TGFβ as a therapeutic target for treatment of this disease.

Disclosures
The authors have no financial conflict of interest.

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